US Army Medical Research and Materiel

Technology Available for Licensing

Real-time PCR Assay for a Unique Chromosomal Sequence of *Bacillus anthracis*

Features and advantages:

- **Demonstrated 100% sensitivity to 45 studied *B. anthracis* strains**
- **Demonstrated 100% specificity against 62 tested *Bacillus* species**
- **Assay is significantly aligned with the *B. anthracis* AMES strain**
- **Assay is homologous to an abhydrolase from *B. anthracis* strain A2012**

This invention is a method to provide a real-time polymerase chain reaction (PCR) assay for the detection of chomosomal DNA from *Bacillus anthracis,* the spore-forming, gram-positive bacterium that causes anthrax. Although the presence of *B. anthracis* can be identified clinically using Gram stain, colony morphology, and various biochemical tests, the methods are time-consuming. Real-time PCR assays are preferred because they are faster, less labor intensive, and add the specificity of a probe.

The *Bacillus* species are genetically indistinguishable from each other. Two chromosomal targets, the *rpoB* and *gyrA* genes, have been published. However, they are not unique to *B. anthracis* and have resulted in false positives. Previous real-time PCR assays identified *B. anthracis* based on the presence of the toxin-coding plasmid (pX01) and capsule-encoding plasmid (pX02) that give *B. anthracis* its virulence. The invention provides a means to identify *B. anthracis* strains that lack pX01 or pX02. The unique chromosomal sequence of the invention relied on a genomic hybridization study that used nucleic acid isolated from *B. cereus* strain 1769 and genomic DNA from the plasmid-cured *B. anthracis* strain Delta ANR-SWS.

The invention could verify the presence of *B. anthracis* independent of plasmid occurrence. It provides a reliable chromosomal assay that was previously lacking.

Patent Status

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